In the Claims

1. (Original) A test set for characterizing substrate specificities of kinases comprising at least two peptide pools, wherein substantially every peptide in each of the peptide pools comprises one phosphorylatable amino acid position, one query amino acid position, at least one anchor amino acid position, and at least one degenerate amino acid position, and wherein:

each peptide of every peptide pool has an identical phosphorylatable amino acid that can be phosphorylated by a kinase at the phosphorylatable amino acid position;

the query amino acid position is at a defined position relative to the phosphorylatable amino acid position within every peptide of every peptide pool but a query amino acid's identity at the query amino acid position is systematically varied from one peptide pool to the next peptide pool within the test set of peptide pools;

each anchor amino acid position is at a defined position relative to the phosphorylatable amino acid position within every peptide of every peptide pool and each anchor amino acid position has an identical anchor amino acid at that anchor amino acid position within every peptide of every peptide pool;

each degenerate amino acid position within every peptide of every peptide pool is occupied by an amino acid from a defined mixture of amino acids; and

the query amino acid position is not adjacent to an anchor amino acid position or the query amino acid position is not adjacent to the phosphorylatable amino acid position in any peptide pool of the test set.

2. (Original) The test set of claim 1, wherein at least one anchor amino acid is arginine.

3. (Original) The test set of claim 1, wherein at least one anchor amino acid is proline.

4. (Original) The test set of claim 1, wherein at least one anchor amino acid is phenylalanine.

5. (Original) The test set of claim 1, wherein an anchor amino acid position is located one position C-terminal to the phosphorylatable amino acid position.

6. (Original) The test set of claim 5, wherein proline is the anchor amino acid at the anchor amino acid position located one position C-terminal to the phosphorylatable amino acid position.

7. (Original) The test set of claim 5, wherein glutamine is the anchor amino acid at the anchor amino acid position located one position C-terminal to the phosphorylatable amino acid position.

8. (Original) The test set of claim 5, wherein arginine is the anchor amino acid at the anchor amino acid position located one position C-terminal to the phosphorylatable amino acid position.

9. (Original) The test set of claim 5, wherein phenylalanine is the anchor amino acid at the anchor amino acid position located one position C-terminal to the phosphorylatable amino acid position.

10. (Original) The test set of claim 1, wherein an anchor amino acid position is located three positions N-terminal to the phosphorylatable amino acid position.

11. (Original) The test set of claim 10, wherein arginine is the anchor amino acid at the anchor amino acid position located three positions N-terminal to the phosphorylatable amino acid position.

12. (Original) The test set of claim 1, wherein every peptide in each of the peptide pools comprises less than four anchor amino acids.

13. (Original) The test set of claim 1, wherein at least one degenerate position in each peptide pool in the test set is occupied by a defined mixture of more than five amino acids.

14. (Original) The test set of claim 13, wherein the defined mixture comprises all natural amino acids.

15. (Original) The test set of claim 13, wherein the defined mixture comprises all natural amino acids except cysteine.

16. (Original) The test set of claim 13, wherein each amino acid's relative abundance in the defined mixture is approximately that amino acid's human proteome relative abundance.

17. (Original) The test set of claim 13, wherein the defined mixture of amino acids comprises proline.

18. (Original) The test set of claim 13, wherein the defined mixture of amino acids comprises arginine.

19. (Original) The test set of claim 1, wherein the test set has at least four peptide pools and each of the four peptide pools have a different query amino acid.

20. (Original) The test set of claim 1, wherein the query amino acid position is two positions N-terminal to the phosphorylatable amino acid position.

21. (Original) The test set of claim 1, wherein the query amino acid position is two positions C-terminal to the phosphorylatable amino acid position.

22. (Original) The test set of claim 1, wherein one query amino acid is proline.

23. (Original) The test set of claim 1, wherein one query amino acid is arginine.

24. (Original) The test set of claim 1, wherein each peptide pool is a soluble mixture of peptides.

25. (Original) The test set of claim 24, wherein substantially every peptide is linked to biotin.

26. (Original) The test set of claim 1, wherein substantially every peptide of every peptide pool is attached to a solid support.

27. (Original) A test set for characterizing substrate specificities of kinases comprising at least two peptide pools, wherein substantially every peptide in each of the peptide pools comprises one phosphorylatable amino acid position, one query amino acid position, and at least one degenerate amino acid position, and wherein:

each peptide of every peptide pool has an identical phosphorylatable amino acid that can be phosphorylated by a kinase at the phosphorylatable amino acid position;

the query amino acid position is at a defined position relative to the phosphorylatable amino acid position within every peptide of every peptide pool but a query amino acid's identity at the query amino acid position is systematically varied from one peptide pool to the next peptide pool within the test set of peptide pools;

each degenerate amino acid position within every peptide of every peptide pool is occupied by an amino acid from a defined mixture of amino acids;

the query amino acid position is not adjacent to the phosphorylatable amino acid position in any peptide pool of the test set.

28. (Original) A test set for characterizing substrate specificities of kinases comprising at least two peptide pools, wherein every peptide in each of the peptide pools comprises one phosphorylatable amino acid position, one query amino acid, at least one anchor amino acid position, and at least one degenerate amino acid position, and wherein:

each peptide of every peptide pool has an identical phosphorylatable amino acid that can be phosphorylated by a kinase at the phosphorylatable amino acid position;

every peptide of every peptide pool has an identical query amino acid but the position of the query amino acid relative to the phosphorylatable amino acid position is systematically varied from one peptide pool to the next peptide pool within the test set of peptide pools;

each anchor amino acid position is at a defined position relative to the phosphorylatable amino acid position within every peptide of every peptide pool and each anchor amino acid position has an identical anchor amino acid at that anchor amino acid position within every peptide of every peptide pool;

each degenerate amino acid position within every peptide of every peptide pool is occupied by an amino acid from a defined mixture of amino acids.

29. (Original) The test set of claim 28, wherein there are at least three peptide pools.

30. (Original) The test set of claim 28, wherein there the query amino acid is arginine.

31. (Original) A binding entity whose binding differentiates between a defined peptide having any one of SEQ ID NO: 76, 79, 81, 82, 87, 89-94, 97, 98, 100, 102, 104, 105, 108, 110, 112, 113, 115, 117, 121, 124, 125, 127-134, 136, 138, 139, 143-145, 148-153, 156, 160, 163-180, 182-194, 196-206, 208-211, 213-216 and the corresponding defined peptide after phosphorylation by PKC-theta, and wherein the binding entity has substantially no binding to a phosphorylated peptide having SEQ ID NO: 229 (WKN-pS-IRH).

32. (Original) The binding entity of claim 31, wherein the binding entity binds less efficiently to the defined peptide than to the defined peptide after phosphorylation by PKC-theta.

33. (Original) The binding entity of claim 32, wherein the binding entity also has substantially no binding to a phosphorylated peptide having SEQ ID NO 230 (RRP-pS-YRK).

34. (Original) The binding entity of claim 32, wherein the defined peptide comprises SEQ ID NO:76 (HVRRRRGTFKRSKLRARD).

35. (Original) The binding entity of claim 32, wherein the defined peptide comprises SEQ ID NO:121 (LRRRSLRRSNSISKSPGP).

36. (Original) The binding entity of claim 32, wherein the defined peptide comprises SEQ ID NO:209 (DKEKSKGSLKRK).

37. (Original) The binding entity of claim 31, wherein the binding entity is a polypeptide or a mixture of polypeptides sharing a similar binding specificity.

38. (Original) The binding entity of claim 31, wherein the binding entity is an antibody, an antibody fragment or a mixture thereof.

39. (Original) The binding entity of claim 31, wherein the binding entity binds more efficiently to the defined peptide than to the defined peptide after phosphorylation by PKC-theta.

40. (Original) The binding entity of claim 31, wherein the defined peptide is part of a protein.

41. (Original) A binding entity whose binding differentiates between a defined phosphorylated peptide having any one of SEQ ID NO:298-347, 349-473 and a non-phosphorylated peptide that differs from the defined peptide by substitution of Ser for the pSer or substitution of a Thr for the pThr, and wherein the binding entity has substantially no binding to a phosphorylated peptide having SEQ ID NO: 229 (WKN-pS-IRH).

42. (Original) The binding entity of claim 40, wherein the binding entity binds more efficiently to the defined phosphorylated peptide than to the defined non-phosphorylated peptide.

43. (Original) The binding entity of claim 41, wherein the defined phosphorylated peptide comprises SEQ ID NO:298.

44. (Original) The binding entity of claim 41, wherein the defined phosphorylated peptide comprises SEQ ID NO:299 or 300.

45. (Original) The binding entity of claim 41, wherein the defined phosphorylated peptide comprises SEQ ID NO:313 or 314.

46. (Original) The binding entity of claim 41, wherein the defined phosphorylated peptide comprises SEQ ID NO:361 or 362.

47. (Original) The binding entity of claim 41, wherein the defined phosphorylated peptide comprises any one of SEQ ID NO:301-310.

48. (Original) The binding entity of claim 41, wherein the defined phosphorylated peptide comprises any one of SEQ ID NO:311-320.

49. (Original) The binding entity of claim 41, wherein the defined phosphorylated peptide comprises any one of SEQ ID NO:321-330.

50. (Original) The binding entity of claim 41, wherein the defined phosphorylated peptide comprises any one of SEQ ID NO:331-342.

51. (Original) The binding entity of claim 41, wherein the defined phosphorylated peptide comprises any one of SEQ ID NO:343-347, 349-362.

52. (Original) The binding entity of claim 41, wherein the defined phosphorylated peptide comprises any one of SEQ ID NO:363-382.

53. (Original) The binding entity of claim 41, wherein the defined phosphorylated peptide comprises any one of SEQ ID NO:383-473.

54. (Original) The binding entity of claim 40, wherein the binding entity binds less efficiently to the defined phosphorylated peptide than to the defined non-phosphorylated peptide.

55. (Original) The binding entity of claim 40, wherein the defined phosphorylated peptide is part of a protein.

56. (Original) A method for characterizing substrate specificities of kinases comprising:

contacting each peptide pool in at least two test sets of peptide pools with ATP and a kinase;

quantifying the amount of phosphorylation in each peptide pool; and

comparing the amount of phosphorylation in each peptide pool with the amount of phosphorylation in at least one other peptide pool;

wherein substantially every peptide in each of the peptide pools comprises one phosphorylatable amino acid position, one query amino acid position, at least one anchor amino acid position, and at least one degenerate amino acid position, and wherein:

each peptide of every peptide pool has an identical phosphorylatable amino acid that can be phosphorylated by a kinase at the phosphorylatable amino acid position;

the query amino acid position is at a defined position relative to the phosphorylatable amino acid position within every peptide of every peptide pool but a query amino acid's identity at the query amino acid position is systematically varied from one peptide pool to the next peptide pool within the test set of peptide pools;

each anchor amino acid position is at a defined position relative to the phosphorylatable amino acid position within every peptide of every peptide pool and each anchor amino acid position has an identical anchor amino acid at that anchor amino acid position within every peptide of every peptide pool; and

each degenerate amino acid position within every peptide of every peptide pool is occupied by an amino acid from a defined mixture of amino acids

57. (Original) The method of claim 56, wherein quantifying the amount of phosphorylation comprises determining a total amount of labeled phosphate incorporated into each peptide pool.

58. (Original) The method of claim 56, wherein quantifying the amount of phosphorylation comprises determining a total amount of phosphorylated peptide in each peptide pool with an antibody specific for a phosphorylated peptide.

59. (Original) The method of claim 56, wherein the method further comprises placing a value for each amount of phosphorylation into a matrix relating amino acid position and amino acid identity with the amount of phosphorylation.

60. (Original) The method of claim 56, wherein the matrix is used to predict preferred substrate peptide sequences for the kinase.

61. (Original) A computer readable medium comprising computer-executable instructions, wherein the computer-executable instructions comprise conversion of input data into quantitative values specifying a preference value for each of a plurality of amino acids at each defined position in a substrate peptide for a kinase, wherein:

the input data comprises sequence and phosphorylation data for a test set of peptides comprising at least two peptide pools, wherein every peptide in each of the peptide pools comprises one phosphorylatable amino acid position, and one query amino acid position, wherein:

each peptide of every peptide pool has an identical phosphorylatable amino acid that can be phosphorylated by a kinase at the phosphorylatable amino acid position;

the query amino acid position is at the defined position relative to the phosphorylatable amino acid position within every peptide of every peptide pool but a query amino acid's identity at the query amino acid position is systematically varied from one peptide pool to the next peptide pool within the test set of peptide pools;

a preference value for a particular amino acid at the defined position is substantially determined from the amount of phosphorylation of the peptide pool wherein that particular amino acid is the query residue and the query position is located at the defined position.

62. (Original) The computer readable medium of claim 61, wherein a ratio between (the preference value for one amino acid) and (the preference value for a second amino acid) is generally proportional to a ratio between (the amount of phosphorylation of the peptide pool in which the first amino acid is the query amino acid) and (the amount of phosphorylation of the peptide pool in which the second amino acid is the query amino acid).

63. (Original) The computer readable medium of claim 61, wherein the difference between (the preference value for one amino acid) and (the preference value for a second amino acid) is generally proportional to a logarithmic transformation of the ratio between (the amount of phosphorylation of the peptide pool in which the first amino acid is the query amino acid) and (the amount of phosphorylation of the peptide pool in which the second amino acid is the query amino acid).

64. (Original) The computer readable medium of claim 61, wherein the instructions further comprise inputting one or more peptide sequences and predicting a likelihood of phosphorylation of the one or more peptide sequences of said kinase.

65. (Original) A method for visual display of amino acid or nucleotide sequence preferences comprising a series of stacks of single letter symbols for amino acids or nucleotides, wherein

each stack represents a position in a peptide or a nucleic acid sequence;

each symbol's height is proportional to the absolute value of a quantitative parameter that is positive for favored amino acids or nucleotides and negative for disfavored amino acids or nucleotides;

each symbol's position within the stack is sorted from bottom to top in ascending value by the quantitative parameter.

66. (Original) A computer readable medium having computer-executable instructions for performing a method of visually displaying amino acid or nucleotide sequence preferences, the method comprising:

representing a position in a peptide or a nucleic acid sequence with a stack of single letter symbols for amino acids or nucleotides; and

displaying a linear array of one or more stacks of letter symbols wherein each letter symbol's height is proportional to the absolute value of a quantitative parameter

that is positive for favored amino acids or nucleotides and negative for disfavored amino acids or nucleotides and wherein each letter symbol's position within the stack is sorted from bottom to top in ascending order by the value of the quantitative parameter.

67. (Original) A computer readable medium having computer-executable instructions of claim 66, wherein the symbols are single letter codes for amino acids.

68. (Original) A computer readable medium having computer-executable instructions of claim 66, wherein the sequence preferences relate to kinase specificity.